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(71) Applicant: INSTITUT PASTEUR
25-28, rue du Docteur Roux
F-75724 Paris Cédex 15(FR)

(72) Inventor: Cole, Stewart
6, rue du Sud
F-92140 Clamart(FR)

(72) Inventor: Streeck, Rolf E.
4 Allee la Fontaine
F-78170 La Celle St.-Cloud(FR)

(74) Representative: Gutmann, Ernest et al,
S.C. Ernest Gutmann - Yves Plasseraud 67, boulevard Haussmann
F-75008 Paris(FR)

(54) Determined DNA sequences derived from a papillomavirus genome, their uses for in vitro diagnostic purposes and the production of antigenic compositions.

(57) The invention concerns DNA fragments derived from the genomic DNA of HPV-33. These fragments are selelected from the group of fragments extending between the nucleotide extremities defined hereafter in relation to the nucleotide-numbering in figs. 1a and 1b respectively:

76– 556
543– 864
867–2811
2728–3808
3326–3575
3842–4079
4198–5611
5516–8091

The invention also relates to the use of these fragments as probes for the detection of HPV in tissue cultures.

EP 0 243 221 A1

DETERMINED DNA SEQUENCES DERIVED FROM A PAPILLOMAVIRUS GENOME, THEIR USES FOR IN VITRO DIAGNOSTIC PURPOSES AND THE PRODUCTION OF ANTIGENIC COMPOSITIONS

The invention pertains to determined DNA sequences derived from a papillomavirus genome, more particularly DNA recombinants, including vectors, modified by such DNA sequences in such manner that, when said DNA recombinants are introduced in suitable host cells in which said DNA recombinants can be replicated, the said DNA sequences can be expressed in the form of the corresponding proteins. The invention further relates to the proteins themselves, which can be purified and used for the production of immunogenic compositions.

The invention pertains more particularly to DNA products of the papillomavirus designated as IP-2 (now re-designated as HPV-33) in the European patent application filed under number 85.402362.9 on November 29, 1985, the contents of which are incorporated herein by reference. A plasmid containing the DNA of said virus has been deposited at the CNCM ("Collection nationale de Culture de Micro-Organismes" of the Pasteur Institute of Paris) under number I-450.

Papillomaviruses are members of the papovavirus family and possess a genome of about 7,900 base pairs (bp) consisting of a covalently closed circular DNA molecule. Human papilloma viruses (HPV) are classified on the basis of their DNA sequence homology (6) and nearly 40 types have now been described. Considerable insight into HPV biology and their involvement in human disease has been attained by the application of the techniques of molecular biology. A possible role for HPVs in human cancer was suspected following the detection of HPV DNA in tumors resulting from the malignant conversion of genital warts (33). The cloning of two HPV genomes, HPV-16 and HPV-18

(3, 11) from cervical carcinomas has further stimulated research in this field of immense socio-economic importance. These viruses were discovered in more than 70 % of the malignant genital tumors examined and in many others HPV-16 related sequences were detected (3, 16, 33). Amongst these is HPV-33 which was recently cloned from an invasive cervical carcinoma using HPV-16 as a probe under conditions of reduced stringency (1). In the present study we have determined the DNA sequence of HPV-33 and describe its relationship to HPV-16. Among the papillomaviruses HPV-33 is unique as it possesses a 78 bp tandem repeat which strongly resembles the enhancer of SV40 (4, 14).

The invention stems from the cloning strategy disclosed hereafter of the genome of HPV-33 which enabled particular DNA sequences to be identified, more particularly those providing hybridization probes, particularly useful for the detection of DNA of papillomaviruses related to HPV-33 in human tissue, whereby positive responses can be related to the possible development in the host of invasive cervical carcinomas.

Reference is hereafter made to the drawings in which the figs concern respectively :

FIGS.1a and 1b.Nucleotide sequence of HPV-33. Position 1 on the circular genome corresponds to a "Hpa-like" sequence found by alignment with HPV-6b.

FIG. 2. Distribution of the major reading frames in the HPV-33 genome. the reading frames were identified by comparison with other HPV sequences and the stop codons are represdented as vertical bars. Also indicated are the locations of unique restriction sites (S, SmaI; E, EcoRV; B2, BglII; B1, BglI) and the likely polyadenylation signals (PA) for the early and late transcripts. In addition to these, 6 other potential PA sites (AATAAA) were detected at positions 862, 1215, 1221, 2666, 5837 and 6239.

FIG. 3. Principle features of the non-coding region. A section of the non-coding region from positions 7500 to 114 is shown. The 78 bp tandem repeats are overlined and those regions resembling the Z-DNA forming element of the SV-40 enhancer are indicated. Potential promoter elements are denoted by stars and the 3 copies of the 12 bp palindrome enclosed between two rows of dots.

Preferred sequences are those which encode full proteins, more particularly and respectively the nucleotidic sequences having the open reading frames referred to in table I hereafter.

The conditions under which the DNA sequence analysis were performed are defined under the heading "MATERIALS AND METHODS" hereafter. The conclusions which were drawn from this sequence analysis appear under the heading "DISCUSSION".

MATERIALS AND METHODS

DNA sequence analysis. The source of HPV-33 sequenced in this study was plasmid p15-5 (1) which consists of a BglII linearized HPV-33 genome cloned in a pBR322 derivative. A library of random DNA fragments (400-800 bp) was prepared in M13mp8 (17) after sonication and end-repair of p15-5, essentially as described previously (28). DNA sequencing was performed by the dideoxy chain termination method (19, 20) with the modifications of Biggin et al. (2). Most of the seQuence was derived in this way although part of the non-coding region was found to be absent or under-represented in the M13 library (> 300 clones). The sequence of this region was obtained directly from p15-5 using the method of Smith (24). Briefly, restriction fragments isolated from 2 "complemenary" M13 clones were used to prime DNA synthesis on templates prepared from p15-5 which had been linearized with a restriction enzyme and then treated with exonuclease III (200 units/pmol DNA for 1 h at 22°C).

Computer analysis. DNA sequences were compiled and

analysed with the programs of Staden (26, 27) as modified by B. Caudron. Optimal alignments of DNA or protein sequences were obtained using the algorithm developed by Wilbur and Lipman (31).

RESULTS AND DISCUSSION

Genomic Arrangement of HPV-33 - The complete 7909 nucleotide sequence of HPV-33, determined by the M13 shotgun cloning/dideoxy sequencing approach, is presented in Fig. 1. On average each position was sequenced 6.5 times. In agreement with the convention for other papillomavirus sequences the numbering begins at a site resembling the recognition sequence for HpaI in the non-coding region.

An analysis of the distribution of nonsense codons (Fig. 2) shows that, as in all other sequenced papillomaviruses, the 8 major open reading frames are located on the same strand. Some features common to HPV-33 and HPV types 1a, 6b and 16 together with the cottontail rabbit papillomavirus and the prototype bovine papillomavirus, BPV-1, (5, 7, 8, 13, 21, 22) include the overlap between the largest open reading frames in the early region, E1 and E2, and the inclusion of E4 within the section encoding E2. Interestingly, the BglII site used in the molecular cloning of HPV-33 is situated within the E1/E2 overlap. Another property common to all papillomaviruses, except BPV-1, is the overlap between the L1 and L2 reading frames. Following L1 is the 892 bp non-coding region which, by analogy with BPV1 (15, 29) undoubtedly contains the origin of replication and various transcriptional regulatory elements. The principal characteristics of the HPV-33 genome are summarized in Table 1.

Nucleotide Sequence Comparison with HPV-16 - HPV-16 is the only other oncogenic papillomavirus, isolated from tumors of the ano-genital region, which has been completely sequenced (22). The gross features of HPV-33 resemble those of HPV-16 except that the E1 reading frame of the latter

is interrupted. All of the coding sequences in HPV-33, except that of E5, are slightly shorter than their counterparts in HPV-16. This may contribute to the fact that its non-coding region, between L1 and E6 (Fig. 2), is 76 bp longer thereby keeping the genomes nearly constant in size.

When the open reading frames were compared pairwise (Table 2) it was found that E1, E2, E6, E7, L1 and L2 displayed between 65-75 % homology whereas those for E4 and E5 were more divergent (about 50 % homology). These findings confirm the heteroduplex analysis performed previously (1). A comparative study (8) of papillomavirus E1 gene products showed that the polypetide consists of an NH_2-terminal segment whose sequence is highly variable, and a COOH-terminal domain of well-conserved primary structure. The longest stretch of perfect sequence homology, 33 nucleotides (positions 1275-1307, Fig. 1) is found near the 5'-end of the E1 reading frame in a region encoding the variable domain of the polypeptide. Several other regions of complete identity (19-28 nucleotides) were detected elsewhere in E1, and also in E2, L2 and L1. As many of these sequences are not found in the genomes of other HPVs, such as HPV-1a and HPV-6b, this raises the possibility that the corresponding oligonucleotides could be produced and used as diagnostic hybridization probes for screening biopsy material from potentially tumorigenic lesions.

Potential Gene Products - The papillomavirus gene products may be divided into those which are believed to play a purely structural role, L1 and L2, and those required for viral propagation and persistence. The results of a comparison of the probable products of the major reading frames from HPVs-33, 16 and 6b are summarized in Table 2. As expected there is strong identity between the ocogenic HPVs-33 and 16, particularly for the proposed E1, E6, E7,

L2 and L1 proteins. When conservative substitutions are included the homology between the two L1 polypeptides increases to 90 % suggesting that the corresponding capsids must be antigenically related. In contrast, significantly weaker homologies were detected when the analysis was extended to include the benign genital wart-forming HPV-6b (Table 2). Comparison of the HPV-16 proteins with those of HPV-6b revealed slightly more homology than was found with HPV-33 suggesting a closer evolutionary relationship.

The non-coding Region - The non-coding region of HPV-33 displays several unique properties and bears only weak resemblance to its homologue in HPV-16. Located between the L1 stop codon and including the putative polyadenylation signal for the late transcripts is a stretch of 223 bp (positions 7097-7320, Fig. 1) unusually rich in T + G (79 %). Contained within this segment are two copies of a 19 bp direct repeat (with one mismatch) and 7 copies of the motif TTGTRTR (where R is A or G). The latter is also found 7 times in the corresponding region of HPV-16 suggesting that it may represent a recognition site for proteins involved in replication. It should be noted that nascent replication forks have been localised in this regiion of the BPV-1 genome (29) and that the origin of replication of the Epstein-Barr virus consists of a family of repeated sequences (32).

A 12 bp palindrome (ACCG....CGGT) that occurs exclusively in the non-coding region of all papillomavirus genomes examined was recently reported by Dartmann et al. (9). Three copies were found in the HPV-33 genome (Fig. 3) and these occupy the same positions in the non-coding region of HPV-16. A role for the palindrome as a possible control site for the early promoter was proposed (4, 9, 15) and indirect support is provided by our finding that the non-coding regions of HPVs, such as HPV-33, do not

display the clustered arrangement of recognition sites for the promoter-specific, activation factor Sp1(12). This is in direct contrast to the situation in another papovavirus, SV40 (12, 14).

The most striking feature of HPV-33 is a perfect 78 bp tandem repeat located 200 bp after the putative origin of replication (Fig. 3). No other repeats of this size or sequence have been described in the genomes of other papillomaviruses. The presumed early promoter for HPV-33 is located about 300 bp downstream from the tandem repeat and the characteristic promoter elements (4) could be identified (Fig. 3). The size, position and arrangement of the 78 bp repeats in the HPV-33 genome suggest that they may function as enhancers of viral transcription. Tandem repeats of 72, 73 and 68 bp have been located near the early promoter of SV40 (4, 14), in the LTR of moloney murine sarcoma virus (10), and in the BK virus genome (23) and shown to enhance transcription from PolII dependent promoters in a cis-active manner. From mutagenesis of the SV40 enhancer (14, 30) and sequence comparisons of characterized transcriptional activators a consensus enhancer sequence was derived. This structure could not be detected in the 78 bp repeat but a potential Z-DNA forming region was uncovered. Z-DNA is believed to attract regulatory molecules to eukaryotic promoters and a Z-DNA antibody binding site has been demonstrated within the SV40 enhancer (18). The sequence to which this antibody binds is also found, albeit with a single mismatch, in the putative HPV-33 enhancer (positions 7520-7527, 7599-7606, Figs. 1, 3).

The proposed HPV-33 enhancer shows no extended sequence homology to the well-characterized enhancers nor to other papillomavirus regulatory regions. However, it has recently been demonstrated that an enhancer-like element is located in the non-coding region of BPV-1 and

that it requires the E2 product for activation (25). These findings support our proposal that the 78 bp tandem repeats could have enhancer function and may indicate that the relatively low homology (Table 2) between the E2 proteins of HPV-33 and 16 reflects a specificity for the corresponding enhancer/regulatory regions.

Tables 1 and 2 which have been referred to in the instant disclosure follow.

TABLE 1. Principai features of the HPV-33 genome

Open Reading Frame	START	FIRST ATG	STOP CODON	mol.wt.
E6	76	109	556 TGA	17 632
E7	543	573	854 TAA	10 825
E1	867	879	2811 TGA	72 387
E2	2728	2749	3808 TAA	40 207
E4	3326	-	3575 TAG	9 452
E5	3842	-	4079 TAA	9 385
L2	4198	4210	5161 TAG	50 539
L1	5516	5594	7091 TAA	55 839

a. Calculated from the first ATG where this exists or from the start of the open reading frame.

TABLE 2. Comparison of HPV proteins[a]

	HPVs		
Protein	33v16	33v6b	16v6b
E6	65(70)	36(51)	37
E7	61(69)	55(60)	56
E1	61(69)	50(60)	53
E2	53(65)	46(58)	45
E4	52(55)	39(46)	48
E5	40(52)	39(43)	33
L2	64(66)	52(58)	53
L1	81(75)	68(69)	71

a - Expressed as % homology after alignment with the program of (31).Values in parenthesis represent % nucleotide sequence homology.

The invention relates more particularly to sequences corresponding to the open reading frames of E6, E7, E1, E2, E4, E5, L2, L1.

The invention pertains also the uses of these sequences as hybridization probes, either those which are useful also for the detection of other papillomaviruses, thus of groups of papillomaviruses - such as probes containing part or all of the open reading frames corresponding to L1 - or those which are more virus - specific, i.e. probes containing part or all of the open reading frame corresponding to.

It also relates to other probes which detect sub-groups of papillomaviruses, particularly probes for the detection of viruses which can be related to major classes of diseases, i.e. viruses associated with tumors. By way of example of one of said probes one should mention that which contains the sequence positionned between nucleotides 1275 and 1307 according to the numbering of the nucleotides in figs. 1A, 1B.

Needless to say that the invention also pertains to all of said DNA sequences, when labelled by a suitable label, i.e. a radioactive enzymatic or immunofluorescent label.

DNAs derived from the viral genome and which carry nucleotides modified by a chemical group which can be recognized by antibodies also form part of the invention. It is well known that such DNAs can be produced by nick-translation in the presence of nucleotides modified accordingly. These DNAs form particularly valuables hybridization probes which, when hybridized to a DNA preparation containing the complementary strand sought, can be detected by the above mentioned antibodies.

The invention also pertains to the diagnostic methods per se. Suitable methods are examplified hereafter.

Several hybridization methods may be used. For example, the spot hybridization method includes, after

denaturation of the DNA, the deposition of an aliquot of the DNA onto film supports (nitrocellulose or Genescreenplus), the hybridization of each film under the usual conditions with the probe, and the detection of the radioactive hybrid by contact exposition of the hybridized film onto radiographic film. Another possibility is replicated culture hyridization which involves agarose gel electrophoresis separation of the DNA fragments resulting from treatment of the DNA by restriction enzymes, the transfer of the fragments after alkaline denaturation onto films (nitrocellulose or Genescreenplus) and their hybridization under usual conditions with different mixtures of probes. The formation of radioactive hybrids is detected again by contact exposition of the hybridization support films onto radiographic film.

For instance the probes of the invention can be used for the detection of the relevant viruses (or DNAs thereof) in preparation consisting of a biopsy of cells obtained by scraping a lesion, or of biopsy sections fixed with Carnoy's mixture (ethanol, chloroform, acetic acid 6:3:1) and included in paraffin.

The above nucleotide sequences can be inserted in vectors, to provide modified vectors which, when introduced in the suitable cell host, are capable of providing for the transcription and, where appropriate, translation of said DNA sequences to produce the corresponding proteins which can then be isolated from cellular extracts of the hosts. Obviously it is within the knowledge of the man skilled in the art to select the appropriate vectors, particularly in relation to the host to be transformed therewith. Vectors consist for instance of plasmids or phages which will be selected according to their recognized capability of replicating in the corresponding procaryotic cells (or yeast cells) and of allowing for the expression of the DNA sequence which they carry.

The invention also relates to DNA recombinants

containing an insert consisting of a DNA sequence corresponding to any of the above-defined open reading frames or of a part thereof, and suitably engineered to allow for the expression of the insert in eucaryotic cells, particularly cells of warm-blooded animal. Suitable DNA recombinants are genetic constructs in which said insert has been placed under the control of a viral or eucaryotic promoter recognized by the polymerases of the selected cells and which further comprise suitable polyadenylation sites downstream of said insert.

By way of example, the invention pertains to DNA recombinants containing any of the above-mentioned open-reading inserts placed under the control of a promoter derived from the genome of the SV40 virus. Such DNA recombinants - or vectors - can be used for the transformation of higher eucaryotic cells, particularly cells of mammals (for instance Vero cells). The invention further pertains to portions of the above identified DNA sequences which, when inserted in similar vectors, are able to code for portions of the corresponding proteins which have immunological properties similar to those encoded by the full nucleotide sequences mentioned above. The similarity of immunological properties can be recognized by the capacity of the corresponding polypeptides produced by the relevant host to be recognized by antibodies previously formed against the proteins produced by the cells previously transformed with vectors containing the above mentioned entire DNA sequences.

It goes without saying that the invention also pertains to any nucleotidic sequence related to the preceding ones which may be obtained at least in part synthetically, and in which the nucleotides may vary within the constrainsts of the genetic code, to the extent where these variations do not entail a substantial modification of the polypeptidic sequences encoded by the so-modified nucleotidic sequences.

It already flows from the preceding discussion that the invention also pertains to the purified proteins or polypeptides themselves as obtainable by the methods discussed hereabove. These polypeptides, when produced in a suitable host, can either be obtained from the cells, for instance after rupturing of their cell walls, or from the culture medium of said cells when excreted in said cell medium, depending on the cell DNA recombinant system which is used. The polypeptide obtained can then be purified by resorting to usual purification procedures. It should be understood that "purified" in the instant context means a level of purity such that, when electrophoresed in SDS-PAGE, the purified proteins yield a single detectable band, say by Western blot.

The viral proteins obtained, more particularly the structural proteins, for instance as a result of the expression of said DNA sequences in E. coli, can be used for the in vitro detection of antibodies against papillomavirus likely to be detected in tissue samples of patients possibly infected with papillomavirus.

Of particular relevance are the genetically engineered proteins having the peptidic sequences which can be deduced from the L1 and L2 open reading frames. Another peptide of interest is the $E6^{*}$ protein (E6 star), the synthesis of which can be induced by splicing and which encoded by a nucleotidic sequence located between nucleotides 229 (donor site) and 404 (acceptor site) of the HPV 33 sequence (see more particularly Fig. 1A), which sites also define the putative splicing sites in the $E6^{*}$ open reading frame of HPV 33. Reference may be had to the publication of Schneider-Gardicke and Schwartz, Embo. J., 5, 2285-2292, as concerns the conditions of the production of such proteins.

These purified polypeptides can in turn be used for the production of corresponding antibodies which can

be used for diagnosing in vitro the presence of viral polypeptides in a biological fluid, particularly in a serum or tissue culture of a patient. Like in the preceding instance, the invention relates to portions of the above defined polypeptides, particularly those which are recognized by the same antibodies or to the contrary are able to elicit in vivo the production of antibodies recognizing the complete proteins.

It must be understood that the inventions relates also specifically to the particular peptides encoded by the DNA regions specifically referred to in the preceding disclosure and which have been found of particular interest.

The invention further concerns host cells transformed with DNA recombinants containing nucleotidic sequences directing the expression of the different peptides mentioned hereabove, and effectively capable to produce said peptides when cultured in an appropriate culture medium.

The invention finally also pertains more particularly to the antibodies themselves which can be obtained from an animal, such as rabbit, immunized in standard manner with said purified polypeptides and/or from hybridomas previously prepared also in any known manner. Of particular inerest are the antibodies (polyclonal and monoclonal antibodies) directed against the strutural proteins. These antibodies are useful for the detection of viral infection. The antibodies which recognize the L1, L2 and E6* proteins of HPV-33 are of particular significance. Antibodies specific of L2 provide diagnostic tools for the in vitro detection of specific viruses sharing with HPV-33 a sequence encoding a similar L2 protein. Antibodies specific to L1 are useful for the detection of the groups of viruses, to which HPV-33 belongs. Antibodies specific to the E6* protein are useful

for the detection of the oncogenic character of the virus causing the abovesaid viral infection.

The invention also relates to intergenic sequences of particular interest, particular the 78 bp sequence. This sequence is of particular interest as a possible insert in eucaryotic vectors, particularly in a position upstream of the promoter and downstream of the site at which transcription of the gene or nucleotide sequence the transcription of which is sought is initiated in the relevant host.

All documents referred to herein are incorporated herein by reference. Particularly these documents can be referred to as concerns the definition of expressions used in this application where appropriate. As such they form part of the present disclosure.

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CLAIMS

1. A DNA fragment consisting of an open reading frame, which DNA fragment is derived from the genomic DNA of HPV-33 and selected from the group of fragments extending between the nucleotide extremities defined hereafter in relation to the nucleotide-numbering in figs. 1a and 1b respectively :

76 - 556
543 - 864
867 - 2811
2728 - 3808
3326 - 3575
3842 - 4079
4198 - 5611
5516 - 8091

2. A DNA fragment encoding a protein, which DNA fragment is derived from the genomic DNA of HPV-33 and selected from the group of fragments extending between the nucleotide extremities defined hereafter in relation to the nucleotide-numbering in figs. 1a and 1b respectively :

109 - 556
573 - 864
879 - 2811
2749 - 3808
3326 - 3575
3842 - 4079
4210 - 5611
5594 - 7091

3. The fragment of claims 1 or 2 which is a cloned fragment.

4. A DNA recombinant, replicable in a cell host, particularly bacteria such as E. coli, which contains an insert containing any of the fragments defined in claims 1 to 3 fused with a DNA foreign thereto.

5. The DNA recombinant of claim 4 which is a vector, wherein said insert is replicable with said vector.

6. The purified peptides obtained from host cells transformed by the vector of claim 5 and in which said insert has been expressed.

7. The antibodies against the peptides of the purified peptides of claim 6.

8. A DNA-hybridization probe for the detection of viral DNA characterized in that it contains a fragment of any of claims 1 to 3 or is formed of the DNA recombinant of claims 4 or 5, or by a part thereof.

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   1  GTAAACTATA ATGCCAAGTT TTAAAAAAGT AGGGTGTAAC CGAAAGCGGT TCAACCGAAA ACGGTGCATA TATAAAGCAA ACATTTTGCA GTAAGGTACT
 101  GCACGACTAT GTTTCAAGAC ACTGAGGAAA AACCACGAAC ATTGCATGAT TTGTGCCAAG CATTGGAGAC AACTATACAC AACATTGAAC TACAGTGCGT
 201  GGAATGCAAA AAACCTTTGC AACGATCTGA GGTATATGAT TTTGCATTTG CAGATTTAAC AGTTGTATAT AGAGAGGGAA ATCCATTTGG AATATGTAAA
 301  CTGTGTTTGC GGTTCTTATC TAAAATTAGT GAATATAGAC ATTATAATTA TTCTGTATAT GGAAATACAT TAGAACAAAC AGTTAAAAAA CCTTTAAATG
 401  AAATATTAAT TAGGTGTATT ATATGTCAAA GACCTTTGTG TCCTCAAGAA AAAAAACGAC ATGTGGATTT AAACAAACGA TTTCATAATA TTTCGGGTCG
 501  TTGGGCAGGG CGCTGTGCGG CGTGTTGGAG GTCCCGACGT AGAGAAACTG CACTGTGACG TGTAAAAACG CCATGAGAGG ACACAAGCCA ACGTTAAAGG
 601  AATATGTTTT AGATTTATAT CCTGAACCAA CTGACCTATA CTGCTATGAG CAATTAAGTG ACAGCTCAGA TGAGGATGAA GGCTTGGACC GGCCAGATGG
 701  ACAAGCACAA CCAGCCACAG CTGATTACTA CATTGTAACC TGTTGTCACA CTTGTAACAC CACAGTTCGT TTATGTGTCA ACAGTACAGC AAGTGACCTA
 801  CGAACCATAC AGCAACTACT TATGGGCACA GTGAATATTG TGTGCCCTAC CTGTGCACAA CAATAAACAT CATCTACAAT GGCCGATCCT GAAGGTACAA
 901  ATGGGGCTGG GATGGGGTGT ACTGGTTGGT TTGAGGTAGA AGCAGTCATA GAGAGAAGAA CAGGAGATAA TATTTCAGAA GATGAGGATG AAACAGCAGA
1001  TGACAGTGGC ACGGATTTAC TAGAGTTTAT AGATGATTCT ATGGAAAATA GTATACAGGC AGACACAGAG GCAGCCCGGG CATTGTTTAA TATACAGGAA
1101  GGGGAGGATG ATTTAAATGC TGTGTGTGCA CTAAAACGAA AGTTTGCCGC ATGTTCACAA AGTGCTGCGG AGGACGTTGT TGATCGTGCT GCAAACCCGT
1201  GTAGAACGTC TATTAATAAA AATAAAGAAT GCACATACAG AAAACGAAAA ATAGATGAGC TAGAAGACAG CGGATATGGC AATACTGAAG TGGAAACTCA
1301  GCAGATGGTA CAACAGGTAG AAAGTCAAAA TGGCGACACA AACTTAAATG ACTTAGAATC TAGTGGGGTG GGGGATGATT CAGAAGTAAG CTGTGAGACA
1401  AATGTAGATA GCTGTGAAAA TGTTACGTTG CAGGAAATTA GTAATGTTCT ACATAGTAGT AATACAAAAG CAAATATATT ATATAAATTT AAAGAGGCCT
1501  ATGGAATAAG TTTTATGGAA TTAGTAAGAC CATTTAAAAG TGATAAAACA AGCTGTACAG ATTGGTGTAT AACAGGATAT GGAATTAGTC CATCAGTAGC
1601  AGAAAGTTTA AAAGTATTAA TTAAACAGCA TAGTTTGTAT ACTCATTTAC AATGTTTAAC TTGCGATAGA GGAATAATAA TATTATTGTT AATTAGATTT
1701  AGGTGTAGCA AAAACAGGTT AACAGTAGCA AAACTAATGA GTAATTTATT ATCAATACCT GAAACATGTA TGGTTATAGA GCCACCAAAA TTACGGAGCC
1801  AAACATGTGC ATTGTATTGG TTTAGAACAG CAATGTCAAA CATTAGTGAT GTACAAGGTA CAACACCTGA ATGGATAGAT AGACTAACTG TTTTACAACA
1901  TAGCTTTAAT GATAATATAT TTGATTTAAG TGAAATGGTA CAGTGGGCAT ATGATAACGA GTTAACGGAC GATAGTGACA TTGCATATTA TTATGCACAA
2001  CTTGCAGATT CAAATAGTAA TGCTGCTGCA TTTTTAAAAA GTAACTCACA AGCAAAAATA GTAAAGGACT GTGGAATAAT GTGTAGACAT TATAAAAAAG
2101  CAGAAAAACG TAAAATGTCA ATAGGACAAT GGATACAAAG TAGATGTGAA AAAACAAATG ATGGAGGAAA TTGGAGACCA ATAGTACAGT TGTTAAGATA
2201  TCAAAACATT GAATTTACAG CATTTTTAGG TGCATTTAAA AAGTTTTTAA AAGGTATACC AAAAAAAAGC TGTATGCTAA TTTGTGGACC AGCAAATACA
2301  GGAAAGTCAT ATTTTGGAAT GAGTTTAATA CAGTTTTTAA AAGGGTGTGT TATATCATGT GTAAATTCTA AAAGTCACTT TTGGTTGCAG CCATTATCAG
2401  ATGCAAAAAT AGGAATGATA GATGATGTAA CGCCAATAAG TTGGACATAT ATAGATGATT ACATGAGAAA TGCGTTAGAT GGAAATGAAA TTTCAATAGA
2501  TGTGAAACAT AGGGCATTAG TGCAATTAAA ATGTCCACCA CTGCTTCTTA CCTCAAATAC AAATGCAGGC ACAGACTCTA GATGGCCATA TTTACATAGT
2601  AGATTAACAG TATTTGAATT TAAAAATCCA TTCCCATTTG ATGAAAATGG TAACCCAGTG TATGCAATAA ATGATGAAAA TTGGAAATCC TTTTTCTCAA
2701  GGACGTGGTG CAAATTAGAT TTAATAGAGG AAGAGGACAA GGAAAACCAT GGAGGAAATA TCAGCACGTT TAAATGCAGT GCAGGAGAAA ATACTAGATC
2801  TTTACGAAGC TGATAAAACT GATTTACCAT CACAAATTGA ACATTGGAAA CTGATACGCA TGGAGTGTGC TTTATTGTAT ACAGCCAAAC AAATGGGATT
2901  TTCACATTTA TGCCACCAGG TGGTGCCTTC TTTGTTAGCA TCAAAGACCA AAGCATTTCA AGTAATTGAA CTACAAATGG CATTAGAGAC ATTAAGTAAA
3001  TCACAGTATA GTACAAGCCA ATGGACATTG CAACAAACAA GCTTAGAGGT GTGGCTTTGT GAACCACCAA AATGTTTTAA AAAACAAGGA GAAACAGTAA
3101  CTGTGCAATA TGACAATGAC AAAAAAAATA CAATGGATTA TACAAACTGG GGTGAAATAT ATATTATAGA GGAAGATACA TGTACTATGG TTACAGGGAA
3201  AGTAGATTAT ATAGGTATGT ATTATATACA TAACTGTGAA AAGGTATATT TTAAATATTT TAAAGAGGAT GCTGCAAAGT ATTCTAAAAC ACAAATGTGG
3301  GAAGTACATG TGGGTGGTCA GGTAATTGTT TGTCCTACGT CTATATCTAG CAACCAAATA TCCACTACTG AAACTGCTGA CATACAGACA GACAACGATA
3401  ACCGACCACC ACAAGCAGCG GCCAAACGAC GACGACCTGC AGACACCACA GACACCGCCC AGCCCCTTAC AAAGCTGTTC TGTGCAGACC CCGCCTTGGA
3501  CAATAGAACA GCACGTACTG CAACTAACTG CACAAACAAG CAGCGGACTG TGTGTAGTTC TAACGTTGCA CCTATAGTGC ATTTAAAAGG TGAATCAAAT
3601  AGTTTAAAAT GTTTAAGATA CAGATTAAAA CCTTATAAAG AGTTGTATAG TTCTATGTCA TCCACCTGGC ATTGGACCAG TGACAACAAA AATAGTAAAA
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FIG.1A

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3701  ATGGAATTGT AACTGTAACA TTTGTAACTG AACAGCAACA ACAAATGTTT TTAGGTACCG TAAAAATACC ACCTACTGTG CAAATAAGTA CTGGATTTAT
3801  GACATTATAA GTGTACATCA CAAGCCAATA TGTGCTGCTA ATTGTATATA ACCATGATAT TTGTTTTTGT ATTATGTTTT ATATTGTTTT TATGCTTATC
3901  CTTATTATTA CGTCCTTTAA TACTTTCCAT TTCTACCTAT GCTTGGTTGC TGGTGTTGGT ATTGCTGCTT TGGGTGTTTG TGGGATCTCC TTTAAAAATT
4001  TTTTTTTGCT ATTTGTTGTT TTTATATTTA CCAATGATGT GTATTAATTT TCATGCACAG CATATGACAC AACAAGAGTA ATGTATATAC ATGTATATAT
4101  TGTTTGTATA TATGTGCACA TGGTGGTGTT TTAACATTGT TGTTGTTATT TTAGTTTTTT TTTTTTGTA TTACTAATAA ATACCTTTAT ATTTTAGCAG
4201  TGTATTATTA TGAGACACAA ACGATCTACA AGGCGCAAGC GTGCATCTGC AACACAACTA TACCAAACAT GCAAGGCCAC AGGCACCTGC CCACCCGATG
4301  TTATTCCTAA AGTGGAAGGA AGTACCATAG CAGATCAAAT TCTTAAATAT GGCAGTTTAG GGGTTTTTTT TGGTGGTTTA GGTATTGGCA CAGGCTCTGG
4401  TTCAGGTGGA AGGACTGGCT ATGTACCTAT TGGTACTGAC CCACCTACAG CTGCAATCCC CTTGCAGCCT ATACGTCCTC CGGTTACTGT AGACACTGTT
4501  GGACCTTTAG ACTCGTCTAT AGTGTCATTA ATAGAAGAAA CAAGTTTTAT AGAGGCAGGT GCACCAGCCC CATCTATTCC TACACCATCA GGTTTTGATG
4601  TTACTACATC TGCAGATACT ACACCTGCAA TTATTAATGT TTCATCTGTT GGGGAGTCAT CTATTCAAAC TATTTCTACA CATTTAAATC CCACATTTAC
4701  TGAACCATCT GTACTACACC CTCCAGCGCC TGCAGAAGCC TCTGGACATT TTATATTTTC TTCCCCTACT GTTAGCACAC AAAGTTATGA AAACATACCA
4801  ATGGATACCT TTGTTGTTTC CACAGACAGT AGTAATGTAA CATCAAGCAC GCCCATTCCA GGGTCTCGCC CTGTGGCACG CCTTGGTTTA TATAGTCGCA
4901  ATACCCAACA CGTTAAGGTT GTTGACCCTG CTTTTTTAAC ATCGCCTCAT AAACTTATAA CATATGATAA TCCTGCATTT GAAAGCTTTG ACCCTGAAGA
5001  CACATTACAA TTTCAACATA GTGATATATC ACCTGCTCCT GATCCTGACT TTCTAGATAT TATTGCATTA CATAGGCCTG CTATTACATC TCGTAGACAT
5101  ACTGTGCGTT TTAGTAGAGT AGGTCAAAAA GCCACACTTA AAACTCGCAG TGGTAAACAA ATTGGAGCTA GAATACATTA TTATCAGGAT TTAAGTCCTA
5201  TTGTGCCTTT AGACCACACC GTGCCAAATG AACAATATGA ATTACAGCCT TTACATGATA CTTCTACATC GTCTTATAGT ATTAATGATG GTTTGTATGA
5301  TGTTTATGCT GACGATGTGG ATAATGTACA CACCCCAATG CAACACTCAT ACAGTACGTT TGCAACAACA CGTACCAGCA ATGTGTCTAT ACCTTTAAAT
5401  ACAGGATTTG ATACTCCTGT TATGTCTGGC CCTGATATAC CTTCCCCTTT ATTTCCCACA TCTAGCCCAT TTGTTCCTAT TTCGCCTTTT TTTCCTTTTG
5501  ACACCATTGT TGTAGACGGT GCTGACTTTG TTTTACATCC TAGTTATTTT ATTTTACGTC GCAGGCGTAA ACGTTTTCCA TATTTTTTTA CAGATGTCCG
5601  TGTGGCGGCC TAGTGAGGCC ACAGTGTACC TGCCTCCTGT ACCTGTATCT AAAGTTGTCA GCACTGATGA ATATGTGTCT CGCACAAGCA TTTATTATTA
5701  TGCTGGTAGT TCCAGACTTC TTGCTGTTGG CCATCCATAT TTTTCTATTA AAAATCCTAC TAACGCTAAA AAATTATTGG TACCCAAAGT ATCAGGCTTG
5801  CAATATAGGG TTTTTAGGGT CCGTTTACCA GATCCTAATA AATTTGGATT TCCTGACACC TCCTTTTATA ACCCTGATAC ACAACGATTA GTATGGGCAT
5901  GTGTAGGCCT TGAAATAGGT AGAGGGCAGC CATTAGGCGT TGGCATAAGT GGTCATCCTT TATTAAACAA ATTTGATGAC ACTGAAACCG GTAACAAGTA
6001  TCCTGGACAA CCGGGTGCTG ATAATAGGGA ATGTTTATCC ATGGATTATA AACAAACACA GTTATGTTTA CTTGGATGTA AGCCTCCAAC AGGGGAACAT
6101  TGGGGTAAAG GTGTTGCTTG TACTAATGCA GCACCTGCCA ATGATTGTCC ACCTTTAGAA CTTATAAATA CTATTATTGA GGATGGTGAT ATGGTGGACA
6201  CAGGATTTGG TTGCATGGAT TTTAAAACAT TGCAGGCTAA TAAAAGTGAT GTTCCTATTG ATATTTGTGG CAGTACATGC AAATATCCAG ATTATTTAAA
6301  AATGACTAGT GAGCCTTATG GTGATAGTTT ATTTTTCTTT CTTCGACGTG AACAAATGTT TGTAAGACAC TTTTTTAATA GGGCTGGTAC ATTAGGAGAG
6401  GCTGTTCCCG ATGACCTGTA CATTAAAGGT TCAGGAACTA CTGCCTCTAT TCAAAGCAGT GCTTTTTTTC CCACTCCTAG TGGATCAATG GTTACTTCCG
6501  AATCTCAGTT ATTTAATAAG CCATATTGGC TACAACGTGC ACAAGGTCAT AATAATGGTA TTTGTTGGGG CAATCAGGTA TTTGTTACTG TGGTAGATAC
6601  CACTCGCAGT ACTAATATGA CTTTATGCAC ACAAGTAACT AGTGACAGTA CATATAAAAA TGAAAATTTT AAAGAATATA TAAGACATGT TGAAGAATAT
6701  GATCTACAGT TTGTTTTTCA ACTATGCAAA GTTACCTTAA CTGCAGAAGT TATGACATAT ATTCATGCTA TGAATCCAGA TATTTTAGAA GATTGGCAAT
6801  TTGGTTTAAC ACCTCCTCCA TCTGCTAGTT TACAGGATAC CTATAGGTTT GTTACCTCTC AGGCTATTAC GTGTCAAAAA ACAGTACCTC CAAAGGAAAA
6901  GGAAGACCCC TTAGGTAAAT ATACATTTTG GGAAGTGGAT TTAAAGGAAA AATTTTCAGC AGATTTAGAT CAGTTTCCTT TGGGACGCAA GTTTTTATTA
7001  CAGGCAGGTC TTAAAGCAAA ACCTAAACTT AAACGTGCAG CCCCCACATC CACCCGCACA TCGTCTGCAA AACGCAAAAA GGTTAAAAAA TAACACTTTG
7101  TGTAATTGTG TTATGTTGTT GTTTTGTTCT GTCTATGTAC TTTGTGTTGT TGTGTTGTGT TGTTGTTTGT TTTTTGTGTA TGTGTTACAA TGTATGTTAT
7201  GTTGTATGTT ACTGTGTTTG TTTTATGTGT ACTTGTTTGT GTGCATGTTC TATGTACTTG TCAGTTTCCT GTTTGTGTAT ATGTTAATAA AACATTGTGT
7301  GTATTTGTTA AACTATTTGT ATGTATGTTA TGTATATGGG TGTACCTATA TGAGTAAGGA GTTGTATTGC TTGCCCTACC CTGCATTGCA ATGTACCTAC
7401  CTTTATTTCC CTATATTTGT AGTACCTACA TGTTTAGTAT TGCTTTACCT TTTGACATAC TAGTGTCCAT ATTGTACAAT TTCCTCCATT TTGTATGCCT
7501  AACCGTTTTC GGTTACTTGG CATACATACC CTATGACATT GGCAGAACAG TTAATCCTTT TCTTTCCTGC ACTGTGTTTG TCTGTACTTG CTGCATTGGC
7601  ATACATACCC TATGACATTG GCAGAACAGT TAATCCTTTT CTTTCCTGCA CTGTGTTTGT CTGTACTTGC TGCATTGACT CATATATACA TGCAGTGCAA
7701  TTGCAAAATA CTTAATTGTA CTAATAGTTT ACACATGCTT TTAGGCACAT ATTTTTACTT TACTTTCAAA CCTTAAGTGC AGTTTTGGCT TACACAATTG
7801  CTTTGTATGC CAAACTATGC CTTGTAAAAG TGAGTCACTA CCTGTTTATT ACCAGGTGTG GACTAACCGT TTTAGGTCAT ATTGGTCATT TATAATCTTT
7901  TATATAATA
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FIG.1B

FIG.2

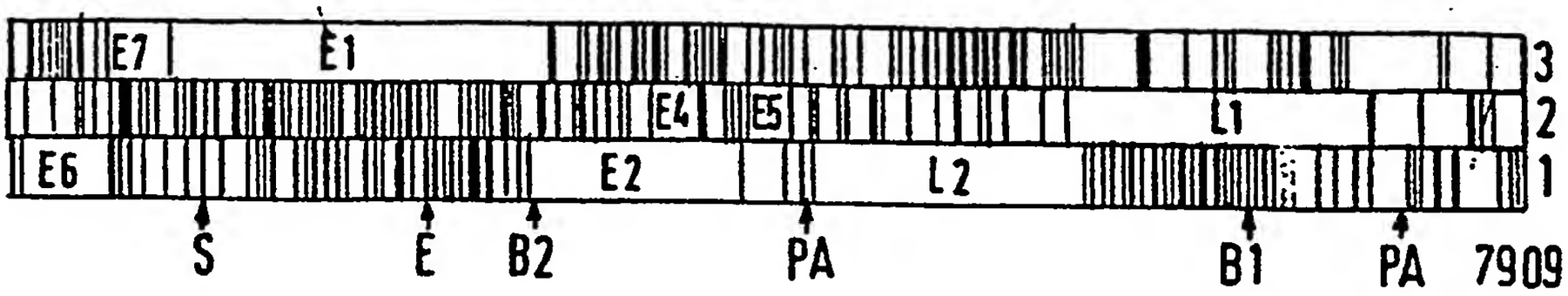
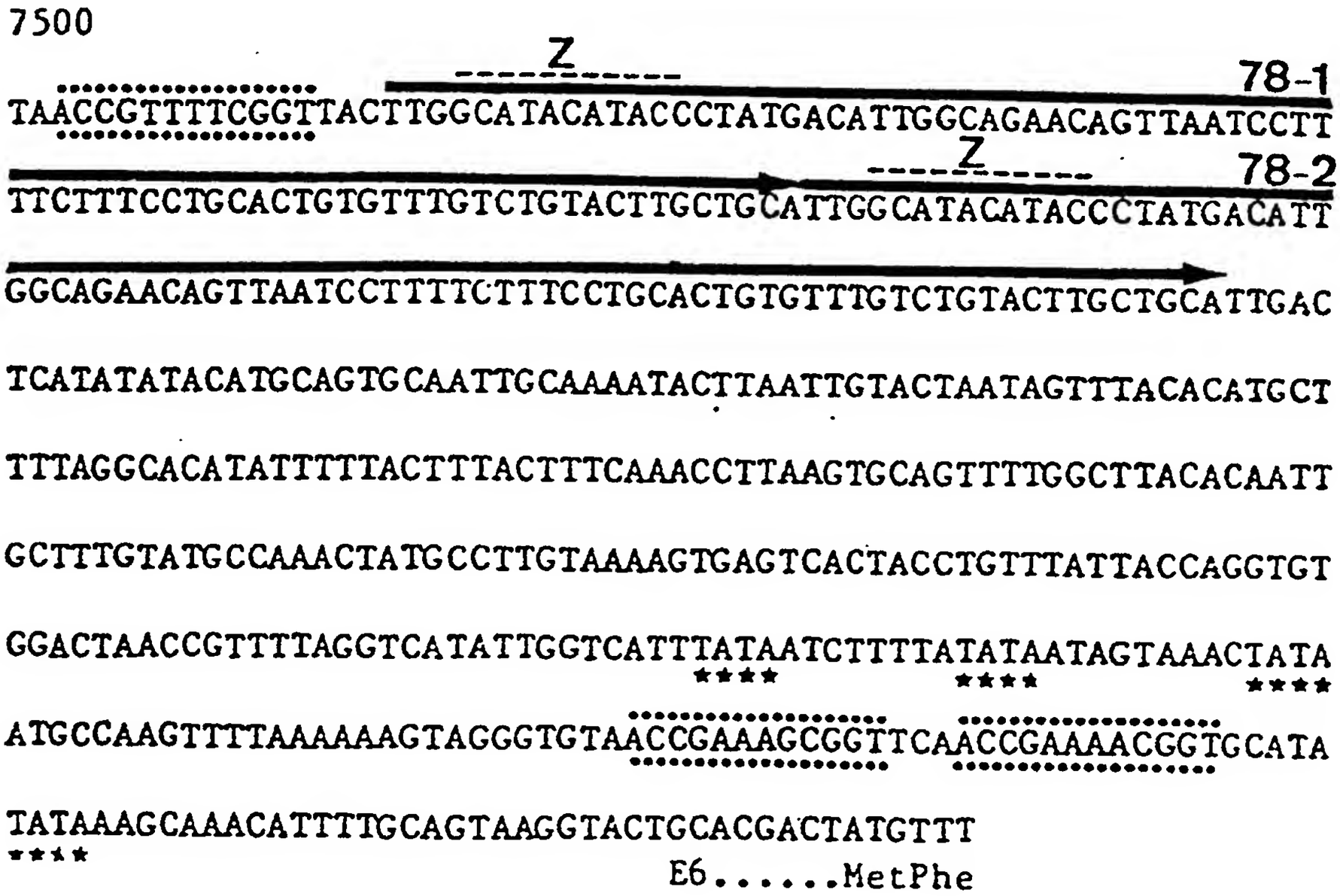

E7
E1
E4
E5
L1
E6
E2
L2
3
2
1
S
E
B2
PA
B1
PA
7909

FIG.3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Z
78-1
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Z
78-2
TTCTTTCCTGCACTGTGTTTGTCTGTACTTGCTGCATTGGCATACATACCCTATGACATT
GGCAGAACAGTTAATCCTTTTCTTTCCTGCACTGTGTTTGTCTGTACTTGCTGCATTGAC
TCATATATACATGCAGTGCAATTGCAAAATACTTAATTGTACTAATAGTTTACACATGCT
TTTAGGCACATATTTTTACTTTACTTTCAAACCTTAAGTGCAGTTTTGGCTTACACAATT
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GGACTAACCGTTTTAGGTCATATTGGTCATTTATAATCTTTTATATAATAGTAAACTATA
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TATAAAGCAAACATTTTGCAGTAAGGTACTGCACGACTATGTTT
E6.....MetPhe

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EUROPEAN SEARCH REPORT

0243221

Application number

EP 87 40 0635

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
Y	UCLA SYMPOSIA ON MOLECULAR AND CELLULAR BIOLOGY, PAPILLOMAVIRUSES: MOLECULAR AND CLINICAL ASPECTS, vol. 32, 1985, pages 391-396, Alan R. Liss, Inc.,; K. SEEDORF et al.: "Human papillomavirus type 16 DNA: Expression of open reading frames in E. coli" * Whole document *	1-8	C 12 N 15/00 A 61 K 39/12 A 61 K 39/42 C 12 Q 1/70
Y	JOURNAL OF VIROLOGY, vol. 57, no. 2, February 1986, pages 688-692; M. KAWASHIMA et al.: "Characterization of a new type of human papillomavirus found in a lesion of Bowen's disease of the skin" * Page 690, especially figure 4B *	1-8	
X,P D	EP-A-0 192 001 (INSTITUT PASTEUR AND INSERM) * Claims 1,7; page 20, last paragraph *	1,2,8	

-/-

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

C 12 N
C 12 Q

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	23-06-1987	CUPIDO M.

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L : document cited for other reasons

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Application number

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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	JOURNAL OF VIROLOGY, vol. 52, no. 3, December 1984, pages 1013-1018, American Society for Microbiology; D. KREMSDORF et al.: "Molecular cloning and characterization of the genomes of nine newly recognized human papillomavirus types associated with Epidermodysplasia Verruciformis" * Whole document *	1-8	
X,P	NATURE, vol. 321, 15th May 1986, pages 246-249; S. BEAUDENON et al.: "A novel type of human papillomavirus associated with genital neoplasias" * Whole document *	1-8	

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

The present search report has been drawn up for all claims

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